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Response of Peritrichous Ciliates in Fouling Communities to Seawater-Accommodated Hydrocarbons

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16. ABSTRACT

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Response of Peritrichous Ciliates in Fouling Communities to Seawater-accommodated Hydrocarbons[1]

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Abstract. Natural assemblages of peritrichous ciliates that develop in the fouling film on glass slides and plastic Petri dishes in both summer and winter were examined in two ecosystems. Fouling in non-stressed communities proceeded to the same biomass and diversity during the winter, albeit more slowly, as that occurring during summertime fouling. The reactions of the ciliates to the water-accommodated fraction (water phase of dispersion) of No. 2 fuel oil ranged from inapparent (0.01 mg/l, 5°C) to almost total exclusion from the fouling film (0.08–0.50 mg/l, 24°C). Intermediate effects included a delay in settlement of two weeks (0.10 mg/l, 5°C), or the stabilization of colonization at a biomass significantly lower than the controls (1.0 mg/l, 5°C). Hydrocarbon stress of stabilized peritrich assemblages consistently resulted in a reduction of biomass. Significant reductions of species diversity occurred only with severe stress. Mild stress resulted in an increase of the "evenness" aspect of diversity, apparently due to disruption of the normal dominance of colonial peritrich species. The withdrawal of hydrocarbon stress caused the biomass and diversity of stressed peritrich assemblages to approach values of the control community within 10 days. The effects of hydrocarbons were not due to toxicity *per se*, but appear to be due to exclusion through migration or avoidance induced by an alteration of the physical-chemical nature of the fouling film by adsorbed hydrocarbons.

Virtually all surfaces submerged in the sea become fouled with organic and inorganic debris, as well as by a variety of microorganisms including bacteria, unicellular algae, fungi, and Protozoa adapted for a sedentary mode of life (Sieburth, 1975). Despite the importance of these microbial communities to such processes as the initiation of food chains on newly submerged surfaces, the breakdown of organic debris, and the regeneration of vital nutrients, the effects of pollution on the processes of microbial fouling are largely unknown.

Ciliated Protozoa often are prominent in planktonic (Porter et al., 1979) and benthic communities (Fenchel, 1967). Protozoa perform a vital role in aquatic ecosystems by serving as a link between bacteria and higher organisms (Sieburth, 1976, 1979). Protozoan predation may be instrumental in keeping bacteria in a state of physiological youth (Johannes, 1965; Lackey, 1967), and may be responsible for a large part of the nutrient regeneration formerly

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attributed to bacteria (Johannes, 1964, 1965). Changes in the structure of a microbial community could have a significant influence on higher trophic levels which depend directly on this community for food, or indirectly for nutrients or vitamins released by these microorganisms (Cairns et al., 1972; Persoone & Uyttersprot, 1975). Peritrichous ciliates are a highly significant component of the fouling film in many aquatic environments (Persoone, 1968; Roos & Trueba, 1977; Sieburth, 1975; Skerman, 1956), and appear to be promising candidates as indicators of stress in microbial fouling communities.

Cultured protists display considerable euroky. This ability may explain the amenability of these species to culture conditions. However, it also may render them more resistant to stress in general (Salt, 1969). The effects of pollution, therefore, must be determined above the species level (Cairns, 1974; Hyland & Schneider, 1976; Persoone & Uyttersprot, 1975). Thus, the "indicator species" concept and the "saprobic system," which relies on exact species identification, have been replaced by changes in protozoan "community structure" as an index of stress. Natural protozoan assemblages have shown responses to stress from heavy metals (Cairns & Dickson, 1970; Ruthven & Cairns, 1973; Spoon & Burbanck, 1967) and chlorine (Cairns & Plafkin, 1975), which transcend successional and seasonal fluctuations.

Among the investigations of the effect of pollution on aquatic ciliates during the past decade are those by Apostol (1973), Carter & Cameron (1973), Cooley & Keltner (1969), Cooley et al. (1973), Dikstein (1969), Gray (1974), Gray & Ventilla (1973), Gregory et al. (1969), Maran et al. (1972), and Tingle et al. (1973). Two attempts to investigate interactions between petroleum hydrocarbons and Protozoa are the studies of Andrews & Floodgate (1974), who noted the ingestion of partially degraded droplets of crude oil during feeding by several species of ciliated Protozoa, and Rogerson & Berger (1981), who studied the effect of crude oil and petroleum degrading microorganisms on the growth of fresh-water and soil Protozoa.

This study investigated both the long- and short-term effects of the water-accommodated fraction (WAF) of No. 2 fuel oil on natural assemblages of peritrichous ciliates. Among the long-term observations were the effect of increasing concentrations of the WAF on the colonization rate of peritrichs, the effect of these hydrocarbons on mature peritrich assemblages which were established in unstressed systems and subsequently subjected to oil stress, and the recovery of stressed assemblages (i.e., return to normalcy upon cessation of stress). Short-term observations included the acute toxicity of the WAF, as well as its effects on substratum selection during the colonization process. Since soluble hydrocarbons finding their way into the marine environment generally are less than 1 mg/l (1 part per million) (Prouse et al., 1976; Van Vleet & Quinn, 1977), only concentrations at or below this level were used.

Materials and Methods

Oil Stressing Systems

Wintertime experiments were conducted in the continuous oil addition flow-through stressing system of the Environmental Protection Agency (EPA),

Narragansett, Rhode Island (Hyland et al., 1977), which employed EPA standard No. 2 fuel oil, purchased from Exxon through the American Petroleum Institute, and had a relatively high aromatic content of 40%. The four different concentrations of hydrocarbons investigated were the background level (control) and additions of approximately 0.01, 0.1, and 1.0 mg/l above control, which yielded actual levels of 0.036 (0.013 S.D.), 0.047 (0.014), 0.09 (0.02), and 0.96 (0.25), respectively. Summertime experiments were conducted in the periodic stressing system of the Marine Ecosystems Research Laboratory (MERL) at the University of Rhode Island (Pilson et al., 1977), which employed three control tanks and three stressed tanks dosed every Monday and Thursday morning with No. 2 fuel oil (O'Neill Oil Service, Peace Dale, Rhode Island) for several months prior to, and continuing through, the period of investigation. The final experimental concentrations ranged from 0.08 to 0.5 mg/l, while the control tanks were consistently below 0.01 mg/l. The seawater-accommodated hydrocarbons, which contain a wide spectrum of hydrocarbons representative of the original hydrocarbon mixture, were obtained from the water phase of dispersion prepared according to Anderson et al. (1974).

Cell Counts

The fouling substrata were chemically cleaned 2.5 × 7.5-cm glass slides that were attached to 30 × 30-cm fiberglass panels, autoclaved, and held at a depth of 0.3 m in the EPA system and at 1.5 m in the MERL tanks. To examine the wet slides immediately upon removal from the tanks, glass coverslips (24 × 60 mm) rimmed with silicone grease were used to make sealed wet mounts which were examined with a Zeiss photomicroscope and phase contrast at 200× magnification. Counts performed within 30 min of sampling recorded the individuals per species of peritrichous ciliates in each of three scans of the length of the coverslip. The peritrich species observed were identified to genus (and sometimes to species) from living specimens, using phase-contrast and Nomarski interference microscopy while consulting references such as Kahl (1935), Kudo (1966), Sommer (1951), Stiller (1939), and Summers (1938). The number of individual cells (indiv.) were recorded for solitary species. Individual cells and the number of colonies (cols.) were enumerated for colonial species.

The triplicate cell counts were converted to ciliates/cm^2 and used to calculate a mean and standard deviation. Cell counts of the peritrich assemblages were used to calculate species diversity by three measurements: (1) number of species; (2) the Simpson index, which is an index of the apportionment of individuals among the species ("evenness") (Sanders, 1968; Simpson, 1949); and (3) the Shannon-Wiener index, which is sensitive to both species number and evenness (Lloyd et al., 1968; Shannon, 1948). The biomass of the peritrich assemblages was determined according to the procedure of Beers et al. (1977), who assumed that 1 cc of ciliate volume was equivalent to 1 g wet weight, that dry weight was 20% of wet weight, and that the carbon content was 40% of the dry weight. The volume of each non-loricate peritrich zooid was determined as the volume of a sphere, while each loricate species

was calculated as the volume of a cylinder. Unidentified peritrich cells (5% of the total) were included in biomass calculations but were not employed in diversity analyses.

Colonization

Wintertime colonization experiments were carried out in the EPA system (15 February 1977 to 22 March 1977) at a water temperature of 2.3 to 9.0°C, and samples were obtained weekly for five weeks. The summertime colonization experiments were performed in the MERL system (18 July 1977 to 1 August 1977) at a water temperature of 22.5 to 25.5°C, and the samples were obtained after 3, 6, 9, 12, and 14 days of substratum exposure.

Hydrocarbon Stress of Mature Peritrich Assemblages

Glass slides were exposed in the control tank of the EPA system (15 February 1977) and in the MERL control tanks (18 July 1977) and transferred in control water to the oil stressed tanks of each system (1.0 mg/l in the EPA system) on 20 April 1977 and 1 August 1977, respectively. Observations were made on these slides prior to transfer, and at frequent intervals following transfer. MERL control tanks 1 and 5 and oil tanks 7 and 9 were used in these stress experiments.

Recovery from Hydrocarbon Stress

Panels which had been exposed in the 1.0 mg/l tank of the EPA system since 15 February 1977 were transferred to the control tank of the EPA system (20 April 1977). Panels exposed in oil tank 9 of the MERL system on 18 July 1977 were transferred to control tank 1 on 1 August 1977. In both systems, samples were taken just prior to and up to nine days following transfer.

Acute Toxicity

To assess the short-term toxicity (48 h) of No. 2 fuel oil on established peritrich assemblages, six 100 × 15-mm plastic Petri dish bottoms were attached to the fouling panels in control tank 1 of the MERL system for 7 days (16–23 March 1977). Lids were replaced under water, and plates were removed filled with water. A total area of 60 mm^2 was examined in each Petri dish at 50× using a Wild M-5 stereomicroscope, and the number of sessile peritrichs per unit area (cells/cm^2) was calculated. After enumeration, the control water in three of the six plates was gently replaced with water taken from the 1.0 mg/l tank of the EPA system, while water in the other three plates was replaced with fresh seawater from control tank 1 of MERL. This water replacement technique has been employed previously without loss of attached peritrichs (Burbanck & Spoon, 1967; Langlois, 1975). The plates were held in an incubator at 18°C and counted after 6, 12, 24, 36, and 48 h. After each count, the water in each dish was replaced with new water from the appropriate tank. The counts included individuals attached to the dishes, free-swimming telotrochs and adult cells detached from their stalks, and cells attached at the water-air interface. Since the latter two groups of cells were

lost at each water renewal, corrected cell counts including these cumulative losses were calculated for the experiment (e.g., the corrected count for the stressed plate at 36 h includes the attached cell count at this time plus the free-swimming and water-air interface cell counts made at 6, 12, 24, and 36 h).

Preferential Colonization

The effect of water-accommodated hydrocarbons in the microbial film on telotroch settlement was observed in plastic Petri dishes containing a thin (1–2 mm) 3% agar surface. Three sets of three dishes each were treated as follows. One set was exposed in the 1.0 mg/l oil tank of the EPA system for 24 h at a depth of 0.3 m to allow hydrocarbon adsorption/absorption and bacterial colonization while excluding peritrichs with 20-μm Nytex mesh. Another set was exposed similarly in control tank 1 of the MERL system for 24 h at a depth of 3 m to allow bacterial colonization without peritrichs in the absence of hydrocarbons. The third set received no pretreatment. All three sets were exposed at a depth of 3 m in control tank 1 of the MERL system and one dish from each set was taken daily for peritrich counts for three days.

Hydrocarbon Retention

Glass slides were attached to a fiberglass fouling panel and submerged in the 1.0 mg/l oil tank of the EPA system at a depth of 0.3 m from 25 May 1977 to 26 July 1977 to allow the development and stabilization of an oil-adapted microbial film. The panel was then removed from the hydrocarbon tank and placed in the control tank of the EPA system. Glass slides were sampled 1 min, 1, 4, 7, 47, 77, and 172 h after transfer to the control tank. Total hydrocarbons were determined by placing the slide in a separatory funnel containing 50 ml of carbon tetrachloride for 5 min (with very gentle periodic agitation) and analyzing the carbon tetrachloride extract for total hydrocarbons on a Grating infrared spectrophotometer (Model 621). The glass slide was removed carefully, dried at 65°C for 24 h, weighed on a Sartorius balance (Series 2600), chemically cleaned in acid dichromate, dried, and reweighed to determine the mass of the film on the slide. The hydrocarbon content of the film on the slide estimated from the carbon tetrachloride extract was converted to milligrams of hydrocarbon per gram of film.

Results

Colonization

The occurrence and abundance of peritrichous ciliate species observed during the colonization experiments in the wintertime EPA and the summertime MERL systems are presented in Tables I and II, respectively. A total of eight species were observed in this study, all eight occurring in the EPA system, and six of the eight in the MERL tanks. Species diversity of peritrich assemblages in both systems is presented in Tables I and II while

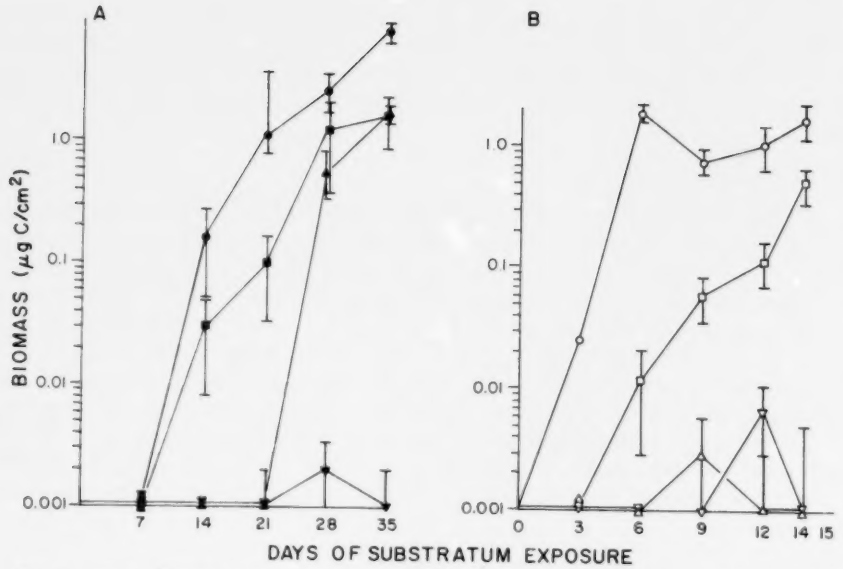

FIG. 1. The influence of water-accommodated hydrocarbons on the biomass of the peritrich assemblages colonizing glass slides. Fig. 1a. EPA system (February–March 1977). ●, control; ■, 0.01 mg/l; ▲, 0.1 mg/l; ▼, 1.0 mg/l. Fig. 1b. MERL system (August 1977). ○, control 1; □, control 5; △, stress 7; ▽, stress 9. Error bars are ±1 standard deviation.

biomass estimates are presented in Fig. 1. Colonization in the control tanks of both systems was the same order of magnitude at the end of the experiments, as indicated by both species number and biomass, despite the large difference in length of time for the two experiments. The elevated summer temperatures undoubtedly account for the immediate colonization observed in the MERL system, and except for an initial lag of one week in the wintertime EPA system, the build up of peritrich biomass was similar for control tanks in both.

A comparison of peritrich colonization for both control and stressed tanks within either system shows that environmental concentrations of oil have a pronounced effect on colonization. This effect is especially evident in the summertime observations in the MERL system. Only one species was observed in the stressed tanks of this system (*Vorticella marina*), and at densities where only a few individuals were observed during the counts. Examination was extended to 25 days in oil tank 7 and no substantial increase was observed during that time. In contrast to the marked suppression of peritrich colonization in the periodically stressed MERL system during summer, a marked suppression occurred only at the highest oil concentration (the 1.0 mg/l tank) in the continuously stressed EPA system during winter.

Stress and Recovery Experiments

The stress and recovery of peritrich assemblages also was examined in both oil stressing systems. Tables III and IV summarize the changes in species abundances and species diversity for control assemblages undergoing hydrocarbon stress. The corresponding changes in biomass for these assemblages are shown in Fig. 2. Changes in the peritrich assemblages in the EPA system due to hydrocarbon stress were due largely to a decreased biomass, while the MERL communities decreased in both biomass and species diversity. Although peritrich biomass decreased almost an order of magnitude in the EPA system, there was very little change in species composition. All three diversity indices investigated showed no decrease during the stress experiment in this system. Peritrich biomass in the MERL system, however, showed decreases of biomass to nearly undetectable limits by the methods used here (approximately 1 cell/cm^2), and with it, a decrease in species diversity. The decrease in biomass in this system was well in excess of two orders of magnitude in eight days.

Recovery of the peritrich assemblages from hydrocarbon stress was merely the reverse of changes induced by stress in nearly the same time span. A comparison of species abundance and diversity for stressed and recovering assemblages in the same system shows reciprocal trends (Tables III, IV). In the EPA system, stress did not affect species numbers, but caused a slight increase or no change in the diversity indices investigated, while recovery caused an overall decrease in these indices. In the MERL system, recovery was accompanied by a marked increase in diversity due to restoration of species eliminated by hydrocarbon stress. The rate of recolonization in recovering assemblages apparently is much faster than the rate of colonization on clean surfaces.

Toxicity Experiment

The fuel oil had no marked toxic effects for peritrichous ciliates. Cell numbers for both control and stressed plates remained close to the starting population for the duration of the experiment; 48-h cell counts for control and stressed plates were 101.4% (11.8 S.D.) and 89.0% (3.6) of the original counts, respectively. The ratio of counts between stressed and control plates did not change if counts of free-swimming peritrichs were omitted. Cell counts for the peritrichs remaining attached to the plates after 48 h were 72.8% of the original population for control plates and 58.0% for stressed plates. The number of free-swimming cells produced during this time (telotrochs and detached adults) was slightly higher in the stressed plates, but was not significantly different from controls. Free-swimming individuals showed no decrease in activity with oil stress, and many reattached at the air-water interface. The marked loss of peritrich cells during the hydrocarbon stress experiments cannot be explained in terms of an acute toxicity of the hydrocarbons for peritrichs.

TABLE I

The influence of water-accommodated hydrocarbons on the occurrence and abundance of species of sessile peritrichous ciliates colonizing glass slides in the EPA oil stressing system (15 February to 22 March 1977)

Days of substratum exposure	Species or diversity index	Cells counts (organisms/cm²)							
		Hydrocarbon supplementation							
		0.0 (control)		0.01 mg/l		0.1 mg/l		1.0 mg/l	
		Cols.[1]	Indiv.[2]	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
7	*Vorticella* sp. 1		3.1[3] ± 3.5		0.8 ± 1.3		—[4]		—
	Simpson Index		0		0		0		0
	Shannon-Wiener Index		0		0		0		0
14	*Vorticella* sp. 1		21.5 ± 4.8		3.1 ± 2.7		—		—
	Vorticella marina		4.6 ± 0.0		15.3 ± 4.8		—		—
	Zoothamnium sp. 1	6.1 ± 3.5	29.1 ± 22.3	0.8 ± 1.3	1.5 ± 2.7	—	—	—	—
	Simpson Index		0.5634		0.3789		0		0
	Shannon-Wiener Index		1.3155		0.9906		0		0
21	*Vorticella* sp. 1		13.0 ± 8.7		1.5 ± 2.7		1.5 ± 1.3		0.8 ± 1.3
	Vorticella marina		17.6 ± 1.3		3.1 ± 2.7		—		—
	Vorticella patellina		41.4 ± 8.3		—		—		—
	Zoothamnium sp. 1	35.3 ± 12.1	164.1 ± 48.8	4.6 ± 2.3	10.7 ± 7.0	—	—	—	—
	Zoothamnium alternans	7.7 ± 7.4	22.2 ± 24.8	15.1 ± 8.7	35.3 ± 19.6	—	—	—	—
	Cothurnia sp.		0.8 ± 1.3		1.5 ± 1.3		—		—
	Simpson Index		0.5589		0.4936		0		0
	Shannon-Wiener Index		1.6497		1.3865		0		0
28	*Vorticella* sp. 1		13.8 ± 8.0		8.4 ± 4.8		26.1 ± 7.4		1.5 ± 1.3
	Vorticella marina		24.5 ± 10.4		14.6 ± 6.6		10.7 ± 2.7		—
	Vorticella patellina		46.0 ± 10.5		—		—		—
	Zoothamnium sp. 1	52.9 ± 9.2	401.0 ± 154.4	26.8 ± 18.6	177.1 ± 153.6	21.5 ± 8.1	82.2 ± 27.1	—	—
	Zoothamnium alternans	59.8 ± 14.4	234.6 ± 43.7	102.0 ± 29.6	235.4 ± 37.2	33.0 ± 4.8	62.9 ± 16.3	0.8 ± 1.3	0.8 ± 1.3

TABLE I

Continued

Days of substratum exposure	Species or diversity index	Cells counts (organisms/cm²)							
		Hydrocarbon supplementation							
		0.0 (control)		0.01 mg/l		0.1 mg/l		1.0 mg/l	
		Cols.[1]	Indiv.[2]	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
	Zoothamnium sp. 2	0.8 ± 1.3	4.6 ± 8.0	—	—	0.8 ± 1.3	6.9 ± 11.9	—	—
	Cothurnia sp.		0.8 ± 1.3		14.6 ± 10.4		18.4 ± 15.9		0.8 ± 1.3
	Vaginicola sp.		0.8 ± 1.3		—		—		—
	Simpson Index		0.5851		0.5692		0.7222		0.6327
	Shannon-Wiener Index		1.5934		1.4466		2.1199		1.5154
35	*Vorticella* sp. 1		6.1 ± 2.7		1.5 ± 1.3		38.3 ± 15.3		0.8 ± 1.3
	Vorticella marina		20.7 ± 2.3		15.3 ± 2.7		2.0 ± 0.0		—
	Vorticella patellina		28.4 ± 11.6		5.4 ± 3.5		—		—
	Zoothamnium sp. 1	99.7 ± 5.8	1,458.2 ± 240.9	21.5 ± 11.4	119.6 ± 85.7	17.6 ± 3.5	216.2 ± 22.6	—	—
	Zoothamnium alternans	79.0 ± 11.8	290.6 ± 93.2	165.0 ± 15.1	562.0 ± 140.2	95.8 ± 2.7	424.7 ± 88.7	—	—
	Zoothamnium sp. 2	6.1 ± 2.7	11.5 ± 4.0	10.7 ± 0.6	53.7 ± 21.4	4.6 ± 2.3	15.3 ± 7.0	—	—
	Cothurnia sp.		6.1 ± 2.7		13.0 ± 5.8		47.5 ± 15.3		0.8 ± 1.3
	Vaginicola sp.		5.4 ± 0.5		0.8 ± 1.3		1.5 ± 2.7		—
	Simpson Index		0.3372		0.4395		0.5842		0.5000
	Shannon-Wiener Index		0.9739		1.3068		1.6095		1.0000

[1] (Cols.) represents the colony count for colonial species.

[2] (Indiv.) is the normal cell count for solitary species as well as the individual zooid count for the colonial species.

[3] Means and ±1 standard deviation are based on triplicate counts.

[4] (—) indicates a cell density below the limit of detection, 0.8 cells/cm².

TABLE II

The influence of water-accommodated hydrocarbons on the abundance of species of sessile peritrichous ciliates colonizing glass slides in the MERL oil stressing system (18 July to 1 August 1977) (Legend as in Table I)

Days of substratum exposure	Species or diversity index	Cell counts (organisms/cm²)							
		Hydrocarbon supplementation							
		Control (#1)		Control (#5)		Oil (#7)		Oil (#9)	
		Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
3	*Vorticella patellina*		20.7 ± 15.9		—		—		—
	Zoothamnium alternans	13.0 ± 3.5	16.1 ± 4.3	—	—	—	—	—	—
	Simpson Index		0.4922		0		0		0
	Shannon-Wiener Index		0.9887		0		0		0
6	*Vorticella* sp. 1		6.9 ± 4.0		—		—		—
	Vorticella marina		9.2 ± 2.3		3.1 ± 1.3		—		—
	Vorticella patellina		299.8 ± 40.0		1.5 ± 1.3		—		—
	Zoothamnium alternans	32.9 ± 3.5	259.1 ± 76.7	—	—	—	—	—	—
	Simpson Index		0.5247		0.4395		0		0
	Shannon-Wiener Index		1.1801		0.9109		0		0
9	*Vorticella* sp. 1		8.4 ± 6.6		—		—		—
	Vorticella marina		17.6 ± 4.8		10.0 ± 5.8		2.3 ± 2.3		—
	Vorticella patellina		112.7 ± 22.6		8.4 ± 2.7		—		—
	Zoothamnium alternans	16.1 ± 6.1	125.0 ± 40.6	1.5 ± 1.3	1.5 ± 1.3	—	—	—	—
	Simpson Index		0.5872		0.5636		0		0
	Shannon-Wiener Index		1.4537		1.3052		0		0

TABLE II
Continued

Days of substratum exposure	Species or diversity index	Cell counts (organisms/cm²)							
		Hydrocarbon supplementation							
		Control (#1)		Control (#5)		Oil (#7)		Oil (#9)	
		Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
12	*Vorticella* sp. 1		7.7 ± 3.5		19.9 ± 5.8		—		—
	Vorticella marina		14.6 ± 2.7		56.0 ± 16.3		0.8 ± 1.3		5.4 ± 2.7
	Vorticella patellina		175.6 ± 70.5		2.3 ± 2.3		—		—
	Zoothamnium alternans	13.8 ± 4.6	92.0 ± 40.9	3.8 ± 1.3	15.3 ± 8.1	—	—	—	—
	Simpson Index		0.5291		0.5686		0		0
	Shannon-Wiener Index		1.3198		1.4768		0		0
14	*Vorticella* sp. 1		13.0 ± 4.8		26.1 ± 5.8		—		—
	Vorticella marina		16.8 ± 8.1		338.1 ± 70.8		—		2.3 ± 4.0
	Vorticella patellina		267.6 ± 88.3		3.8 ± 3.5		—		—
	Zoothamnium alternans	20.7 ± 4.6	219.3 ± 41.4	3.1 ± 2.7	14.6 ± 13.9	—	—	—	—
	Cothurnia sp.		10.0 ± 5.3		1.5 ± 1.3		—		—
	Vaginicola sp.		3.8 ± 0.2		—		—		—
	Simpson Index		0.5727		0.2190		0		0
	Shannon-Wiener Index		1.4727		0.7020		0		0

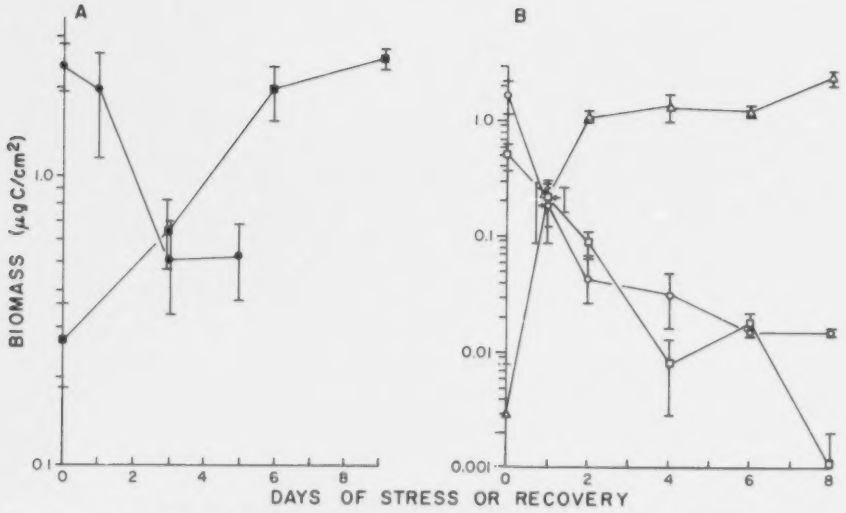

FIG. 2. Comparison of the rates of change in peritrich biomass during both hydrocarbon stress and recovery from stress. Fig. 2a. EPA system (April 1977). ●, stress; ■, recovery. Fig. 2b. MERL system (August 1977). ○, established in control tank 1, stressed in 9; □, established in control tank 5, stressed in 7; △, established in stress tank 9, recovery in control tank 1. Error bars = ±1 standard deviation.

Preferential Colonization Experiment

An agar surface containing hydrocarbons and a bacterial film had a colonization density intermediate between a sterile agar surface and an agar surface with a bacterial film but without hydrocarbons. Cell densities for these three treatments were 169.5 (9.3 S.D.), 130.0 (32.2), and 215.0 (13.0) cells/cm², respectively, after two days of exposure. Differences in settlement after three days were not significant. These results indicate that the presence of the hydrocarbons acts to reduce the colonization density of peritrichs relative to fouled controls, and suggest that peritrichs are excluded from a fouling film containing fuel oil. When the hydrocarbon stress was removed from a "damaged" community in the recovery experiment, the community soon recolonized, indicating that the modification of the surface in the fouling film was transient.

Hydrocarbon Retention Experiment

A significant loss of petroleum hydrocarbons from the fouling film occurs in a very short time once the film is removed from stressed conditions (Fig. 3). Approximately 65% of the hydrocarbons present in the film at initial sampling were purged from the film after 24 h exposure in the control tank. These data are in agreement with results of the recovery experiments, which showed that recovery to near-control conditions occurs in a very short time. It also supports the hypothesis that the activity of petroleum hydrocarbons in the

TABLE III

The influence of hydrocarbon stress and recovery from hydrocarbon stress on populations of peritrichous ciliates, EPA system (Legend as in Table I)

Species or diversity index	Cell counts (organisms/cm²)							
	Days of stress in 1.0 mg/tank							
	Before stress		1		3		5	
	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
(A) *Vorticella* sp. 1		24.5 ± 4.8		8.4 ± 2.7		6.9 ± 2.3		13.0 ± 3.5
Vorticella marina		80.5 ± 18.4		103.5 ± 64.8		3.8 ± 1.3		13.0 ± 4.8
Vorticella patellina		287.5 ± 51.8		257.6 ± 91.9		50.6 ± 22.2		79.0 ± 21.9
Zoothamnium alternans	101.2 ± 14.4	539.7 ± 120.8	146.4 ± 33.0	407.9 ± 86.1	112.7 ± 18.3	207.8 ± 42.2	65.9 ± 17.3	76.7 ± 24.2
Zoothamnium sp. 2	3.8 ± 1.3	19.9 ± 9.6	—	—	—	—	—	—
Cothurnia sp.		—		—		1.5 ± 1.3		—
Pyxicola socialis		6.1 ± 2.7		—		2.3 ± 4.0		5.4 ± 5.3
Simpson Index		0.5845		0.5971		0.3849		0.6432
Shannon-Wiener Index		1.5855		1.4741		1.0695		1.7348

Diversity index	Cell counts (organisms/cm²)							
	Days of recovery in control tank							
	Before recovery		3		6		9	
	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
(B)								
Vorticella sp. 1		13.7 ± 6.1		123.4 ± 44.7		59.8 ± 17.9		42.9 ± 19.6
Vorticella marina		43.7 ± 19.6		25.3 ± 8.0		61.3 ± 12.7		34.5 ± 6.1
Vorticella patellina		—		7.7 ± 7.4		167.1 ± 57.5		184.8 ± 21.9
Zoothamnium alternans	6.1 ± 1.3	8.4 ± 1.3	160.2 ± 32.2	374.9 ± 80.0	236.9 ± 16.6	798.1 ± 52.7	229.2 ± 7.0	1,245.1 ± 41.4
Zoothamnium sp. 2	20.7 ± 4.6	28.4 ± 8.1	—	—	0.8 ± 1.3	0.8 ± 1.3	—	—
Cothurnia sp.		6.9 ± 4.0		1.5 ± 1.3		0.8 ± 1.3		2.3 ± 2.3
Pyxicola socialis		14.6 ± 5.8		9.2 ± 10.5		39.1 ± 6.1		32.2 ± 10.5
Simpson Index		0.7665		0.4670		0.4695		0.3318
Shannon-Wiener Index		2.2803		1.2708		1.3972		1.0129

(A) Hydrocarbon stress of peritrich assemblages established on glass slides exposed in the control tank.
(B) Recovery of peritrich assemblages established on glass slides exposed in the 1.0 mg/l tank.

TABLE IV

The influence of hydrocarbon stress and recovery from hydrocarbon stress on populations of peritrichous ciliates, MERL system (Legend as in Table I)

Species or diversity index	Cell counts (organisms/cm²)											
	Days of stress/recovery											
	Before stress		1		2		4		6		8	
	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
(A) *Vorticella* sp. 1		26.1 ± 5.8		26.8 ± 7.0		16.1 ± 2.3		2.3 ± 2.3		1.5 ± 1.3		2.3 ± 2.3
Vorticella marina		338.1 ± 70.8		118.1 ± 8.7		60.0 ± 14.0		6.1 ± 3.5		13.0 ± 2.7		—
Vorticella patellina		3.8 ± 3.5		—		—		—		—		—
Zoothamnium alternans	3.1 ± 2.7	14.6 ± 13.9	5.4 ± 1.3	34.5 ± 28.0	—	—	—	—	—	—	—	—
Cothurnia sp.		1.5 ± 1.3		3.1 ± 1.3		0.8 ± 1.3		—		—		—
Vaginicola sp.		—		3.1 ± 1.3		—		—		—		—
Simpson Index		0.2190		0.5391		0.3453		0.3977		0.1855		0
Shannon-Wiener Index		0.7020		1.4666		0.8166		0.8469		0.4798		0
(B) *Vorticella* sp. 1		13.0 ± 4.8		88.2 ± 24.6		86.6 ± 41.3		9.2 ± 4.6		4.6 ± 2.3		4.6 ± 2.3
Vorticella marina		16.8 ± 8.1		32.2 ± 10.0		11.5 ± 4.0		—		—		—
Vorticella patellina		267.6 ± 88.3		19.6 ± 15.3		—		—		—		—
Zoothamnium alternans	20.7 ± 4.6	219.3 ± 41.4	3.1 ± 1.3	9.2 ± 4.6	—	—	—	—	—	—	—	—

TABLE IV
Continued

Species or diversity index	Cell counts (organisms/cm²)											
	Days of stress/recovery											
	Before stress		1		2		4		6		8	
	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.	Cols.	Indiv.
Cothurnia sp.		10.0 ± 5.3		—		—		2.3 ± 2.3		—		—
Vaginicola sp.		3.8 ± 0.2		—		—		—		—		—
Simpson Index		0.5727		0.5829		0.2070		0.3200		0		0
Shannon-Wiener Index		1.4727		1.5583		0.5213		0.7219		0		0
(C)*Vorticella* sp. 1		—		—		6.9 ± 0.0		6.1 ± 2.7		13.8 ± 6.1		18.4 ± 9.2
Vorticella marina		2.3 ± 4.0		6.1 ± 4.8		10.7 ± 3.5		37.6 ± 12.7		56.7 ± 21.7		128.0 ± 9.3
Vorticella patellina		—		39.9 ± 17.2		198.6 ± 18.6		240.0 ± 57.8		217.7 ± 112.9		424.0 ± 58.2
Zoothamnium alternans	—	—	—	—	0.8 ± 1.3	2.3 ± 4.0	2.3 ± 4.0	11.5 ± 19.9	3.1 ± 3.5	16.1 ± 22.2	6.1 ± 1.3	31.4 ± 13.5
Cothurnia sp.		—		—		—		—		3.1 ± 3.5		'.6 ± 2.3
Vaginicola sp.		—		—		0.8 ± 1.3		—		—		—
Simpson Index		0		0.2300		0.1764		0.3208		0.4596		0.4629
Shannon-Wiener Index		0		0.5645		0.5976		0.9195		1.2931		1.2622

(A & B) Hydrocarbon stress of peritrich assemblages established on glass slides exposed in tanks 1 and 5 (control).
(C) Recovery of peritrich assemblages established on glass slides exposed in tank 9 (oil stressed).

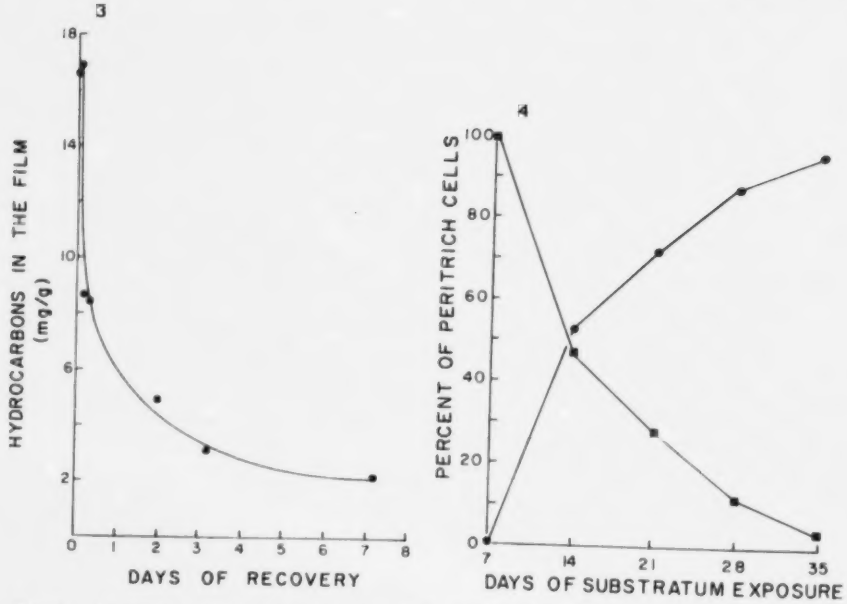

FIG. 3. Decrease in the hydrocarbon concentration of a microbial film established in the 1.0 mg/l EPA tank when transferred to the control tank (July 1977).

FIG. 4. The reciprocal relationship of solitary and colonial species of peritrichous ciliates during colonization (EPA control water, February–March 1977). ■, solitary cells; ●, colonial cells.

preferential fouling experiment, although important, probably is very short-lived.

DISCUSSION

Concentrations of hydrocarbons which one commonly encounters in the estuarine environment can have a serious impact on natural assemblages of peritrichous ciliates. Observations on the colonization process while under hydrocarbon stress, and its effect on stabilized peritrich assemblages, showed changes ranging from decreases in biomass without severely affecting species composition, to the virtual elimination of peritrichs from the primary fouling film. Such changes, however, do not appear to be brought about necessarily by toxicity of hydrocarbons to these Protozoa. From the data obtained, the decrease in peritrich abundance apparently is due mostly to inhibition of peritrich settlement, presumably caused by chemical-physical alteration of the fouling film by adsorbed hydrocarbons. The results obtained in the experiments on peritrich community structure, however, do not immediately suggest this conclusion.

The effect of hydrocarbon stress on colonization of glass slides by peritrichs

showed striking results in both systems. In the EPA system during the winter, hydrocarbon concentration of 1.0 mg/l caused almost complete exclusion of peritrichs over the 35-day examination period. Likewise, the colonization experiment performed in the MERL system during the summer showed severe effects as well, and at substantially lower effective hydrocarbon concentrations (0.08–0.50 mg/l). Although this may have been due in part to the different fuel oils employed, it is more probable that increased temperature during the summer intensified the influence of the hydrocarbons in the MERL tanks.

Although our data appear to indicate a high degree of species toxicity for peritrich assemblages associated with the WAF of No. 2 fuel oil, the results of the toxicity experiment indicate that toxicity is not the primary factor determining changes in peritrich assemblages exposed to hydrocarbon stress. If acute toxicity is associated with the fuel oil, it is so slight (~10% after 48 h) as to be insignificant, compared with the large decreases in biomass observed in the mature community stress experiments (Fig. 2).

If toxicity is discounted as an important factor in explaining our results, an alternative interpretation might be that hydrocarbons produce a surface which is undesirable for peritrich settlement. During the species toxicity experiments, large numbers of cells were released from the surface as free-swimming cells (telotrochs and adult cells detached from the surface). If recolonization is reduced in the stressed films, individuals lost through predation or stalk abandonment would not be replaced by telotroch settlement. The net effect would be a decrease in peritrich biomass that could be accomplished without acute toxicity. The exclusion hypothesis resolves the apparent contradiction between decreases in peritrich biomass observed during the mature peritrich assemblage stress experiments, and lack of toxicity of hydrocarbons for peritrichs. Increased stalk abandonment in the oil-stressed tanks would further increase the opportunity for migration out of the microbial fouling community. Natural or accelerated migration out of the affected area can account for the decreases in biomass observed within the time span of several days.

Recovery of stressed peritrich assemblages to near-control levels in both biomass and species diversity took place rapidly after cessation of hydrocarbon stress. In fact, colonization rates for "recovering" assemblages were faster than rates for clean surfaces. Accelerated rates of recolonization also have been shown for fresh-water protozoan communities exposed to detergent enzymes (Cairns et al., 1971). The combination of a rapid hydrocarbon loss from the recovering film, and the presence of a well-established bacterial flora may account for the rapid recovery of the peritrich assemblages after removal from a hydrocarbon-stressed environment.

Accelerated recolonization is evident in the results of the preferential colonization experiment and the hydrocarbon retention experiment. Results of the latter experiment showed a decrease of approximately 65% of total hydrocarbons in the fouling film within 24 h. In the preferential colonization experiment, an agar surface exposed in the 1.0 mg/l oil tank of the EPA

system, and later allowed to foul in control tank 1 of the MERL system, showed a colonization rate which was greater than a sterile agar surface but less than an agar surface which had been exposed to control seawater.

Hydrocarbon stress of mature peritrich assemblages in the EPA system resulted in increases in the Simpson and Shannon-Wiener indices, while recovery gave the opposite result (Table III). Peritrich assemblages are usually dominated numerically by colonial species, due to reduced competition for settlement space (Nusch, personal communication). During the colonization process in the control waters of the EPA system, there was a marked replacement of the initial colonizing solitary cells by colonial forms (Fig. 4). The introduction of stress resulted in a reduction of the normal dominance of colonial species in the peritrich assemblage. Prior to stress, two colonial species comprised 59% of the peritrich cells, while after five days of stress the contribution had dropped to 41%. During the recovery process, the colonial contribution rose from 25% to 81% (Table III). The reversal in dominance by colonial species in the peritrich assemblages caused the observed changes in the "evenness" aspect of the community, as shown by the Simpson and Shannon-Wiener indices.

The possible effects which water-accommodated hydrocarbons might have on peritrich telotrochs include: (1) toxicity; (2) prevention of metamorphosis; (3) disruption of chemosensory perception, or production of a negatively chemotactic surface; and (4) physical alteration of the surface, making attachment difficult or unachievable. Our results lend no support to (1) and (2), but rather suggest that chemical or physical alteration of the fouling surface may be the major inhibitor of peritrich settlement.

Data on the response of protists to chemical cues are rare. The settling and attaching response of a heterotrophic marine dinoflagellate *Crypthecodinium cohnii* to chemicals impregnating an agar gel has been studied (Hauser et al., 1975; Levandowsky et al., 1975). The results suggest that the chemical nature of the substratum can be an important factor in settlement behavior. Observations on peritrichs also support this hypothesis. The inhibitory activity of the polyphenols of brown algae (Sieburth, 1968) appears to cause selective colonization of rockpool algae by the peritrich *Vorticella marina* (Langlois, 1975). Also, colonization of specific crustacean species by certain species of sessile peritrichs suggests some form of chemical recognition of host by the peritrichs (Fenchel, 1965; Herman & Mihursky, 1964; Hirche, 1974; Turner et al., 1979).

Physical factors also may be involved in settlement inhibition. Wettability of a surface has been shown to influence adhesion in biological systems (Baier et al., 1968). In this connection, attachment of bacteria in seawater has been correlated with the critical surface tensions of various substrata (Dexter et al., 1975). Changes in the wettability of the glass slides might be brought about by hydrocarbons adsorbing to surfaces in the oil-stressed tanks. Thus, adsorbed hydrocarbons might adversely affect peritrich settlement by an alteration in the physical characteristics of the surface.

The precise manner in which hydrocarbons affect peritrich settlement is

not yet known, and may be a combination of both chemical and physical alteration of the fouling film. Our data suggest that low-level hydrocarbon concentrations may be of considerable concern in some marine systems, such as activated sludge wastewater treatment systems, where peritrichs play a major role in the microbial fouling community.

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